the A&E departments, shut the hospitals, close the operating theatres due to a lack of beds, and break every target in every acute hospital service. Ironically, it will continue its lonely position as the "Cinderella" of medicine.

Thorax 2002;57:753–754

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REFERENCES

1 British Thoracic Society. *The burden of lung disease.* London: British Thoracic Society, 2001.
2 Anderson HR, Esmail A, Hollowell J, *et al. Epidemiologically based needs assessment: lower respiratory disease.* London: Department of Health, 1994.
3 Department of Health. *Annual trust returns.* London: Department of Health, 1999.
4 Lopez AD, Murray CL. The global burden of disease. *Nature Med* 1998;4:1241–3 .
5 Patel IS, Seemungal TAR, Wilks M, *et al.* Relationship between bacterial colonisation and the frequency, character and severity of COPD exacerbations. *Thorax* 2002;57:759–64.
6 O'Brien C, Guest PJ, Hill SL, *et al.* Physiological and radiological characterisation of patients diagnosed with chronic obstructive pulmonary disease in primary care. *Thorax* 2000;55:635–42.
7 Seemungal TA, Harper-Owen R, Bhowmik A, *et al.* Symptoms, inflammatory markers and respiratory viruses in acute exacerbations and stable COPD. *Am J Respir Crit Care Med* 2001;164:1618–23.
8 Walsh EE, Falsey AR, Hennessy PA. RSV and other virus infections in persons with chronic cardiopulmonary disease. *Am J Respir Crit Care Med* 1999;160:791–5.
9 Hogg JC. Childhood viral infection and the pathogenesis of asthma and chronic obstructive lung disease. *Am J Respir Crit Care Med* 1999;160:26–8.
10 Omenaas E, Bakke P, Eide GE, *et al.* Serum respiratory virus antibodies: predictor of reduced one-second forced expiratory volume (FEV1) in Norwegian adults. *Int J Epidemiol* 1996;25:134–41.
11 Nelson JK, Sheils MD, Stewart MC *et al.* Seroprevalence of respiratory virus infections in an infant population with a multiantigen fluorescence immunoassay using heel-prick blood samples collected on filter paper. *Pediatr Res* 1999;45:799–802.
12 Gilmour PS, Rahman I, Hayashi S, *et al.* Adenoviral E1A primes alveolar epithelial cells to PM(10)-induced transcription of interleukin-8. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L598–606.
13 Kidney JC, Proud D. Neutrophil transmigration across human airway epithelial monolayers: mechanism and dependence on electrical resistance. *Am J Respir Cell Mol Biol* 2000;23:389–95.
14 Soler N, Ewig S, Torres A, *et al.* Airway inflammation and bronchial microbial patterns in patients with stable chronic obstructive pulmonary disease. *Eur Respir J* 1999;14:1015–22.
15 Staunton DE, Merluzzi VJ, Rothlein R, *et al.* A cell adhesion molecule, ICAM-1, is the major surface receptor for rhinoviruses. *Cell* 1989;56:849–53.

Gene expression profiling

Gene expression profiling: good housekeeping and a clean message

R C Chambers

Microarray technology offers us the means of monitoring gene expression on a scale which was hard to envisage only a few years ago.

There is no doubt that gene expression studies based on evaluating mRNA levels for single or multiple genes of interest in human lung biopsy tissue have had a major impact on our understanding of the molecular mechanisms underlying respiratory disease. The recent advent of microarray technology has added further impetus to the central paradigm that mRNA quantification in lung tissue can shed light on pathogenesis and identify new targets for therapeutic intervention. This technology is now so advanced that it allows the parallel monitoring of entire genomes using microarrays with a surface area equivalent to just a few square centimetres and as little as 5 μg RNA starting material.

Since its first application in the mid 1990s,[1] microarray technology has been applied to all aspects of biomedical research with over 60 papers in respiratory research alone. It has been successfully used for the classification and molecular diagnosis of lung cancer,[2] the identification of potential target genes for therapeutic intervention in idiopathic pulmonary fibrosis,[3] mechanistic studies in animal models of asthma[4] and pulmonary fibrosis,[5] and for profiling lung development.[6] Global expression profiling of cellular responses in vitro has provided new insights into the transcriptional programs involved in cytokine signalling,[7] growth arrest and apoptosis,[8] and it is already enabling us to understand the operation of functional gene networks.

MICROARRAY PLATFORMS

Although a number of microarray platforms have been developed, microarrays come in two basic formats. Complementary DNA (cDNA) arrays usually contain polymerase chain reaction (PCR) products generated from cDNA libraries or clone collections, spotted onto glass slides or nylon membranes. Expression values are based on the competitive hybridisation of two samples being directly compared following the incorporation of two fluorescent dyes (Cy3 and Cy5) on a single array. In contrast, oligonucleotide arrays (for example, Affymetrix GeneChips) contain relatively short sequences (20-mers) synthesised onto silicon wafers in situ by photolithography or arrayed as pre-synthesised oligonucleotides onto glass slides. The final target consists of biotin labelled cRNA and each sample is hybridised to a separate array. Hybridisation is detected by staining with a streptavidin-phyocoerythrin conjugate followed by confocal fluorescence laser scanning. The advantage of oligonucleotide arrays is that they contain multiple validated probe sequences for each gene and mismatch control sequences to allow correction of non-specific hybridisation signals. In contrast, cDNA arrays usually consist of user defined probe sequences but allow a much greater degree of flexibility and are generally cheaper as slide printing can be performed in house.

EXTRACTING BIOLOGICAL MEANING

Managing and mining the huge amount of data generated by microarray experiments remains a major challenge for most users. Although this side of microarray analysis is still considered a major bottleneck, help is at hand via a plethora of online data mining, clustering, and analysis tools. In fact, most of the best tools are available to academic users as freeware upon request. A detailed description of these tools is beyond the scope of this editorial. However, Gene Express (http://www.thoracic.org/geneexpress/), a new column edited by Naftali Kaminski and hosted by the ATS website, is a valuable resource aimed specifically at lung researchers and an excellent route to other sites of interest.

Despite its growing use in both academia and industry, microarray experiments are still considered by many

as nothing more than a sophisticated fishing trip. This is because microarray analysis challenges the traditional hypothesis driven method of investigation and shifts the emphasis towards hypothesis generation. Investigators are then faced with what is probably the greatest challenge—namely, the extraction of biological meaning from microarray data and the prioritisation of candidate genes for follow up. Faced with hundreds of possibilities, it is not surprising that investigators have, in the past, tended to focus on genes they recognise and can integrate into a reasonable hypothesis regarding their likely role in the disease process. Fortunately, the need to address these limitations of microarray analysis is fuelling the rapid development of novel computational tools. This includes unbiased scoring methods for identifying the most meaningful and informative genes in microarray experiments. One such tool has recently been applied to great effect to funnel and prioritise candidate genes for follow up in expression studies of human lung biopsy material from patients with pulmonary fibrosis.[3] Used in combination with computational tools which allow the visualisation of gene expression data on maps representing biological pathways (for example, GenMAPP at http://gladstone-genome.ucsf.edu/) and programs based on artificial neural networks which can be trained to recognise signature expression profiles,[9] these tools are likely to significantly accelerate our understanding of the molecular basis of disease.

VALIDATION OF MICROARRAY DATA

Although microarray technology is improving rapidly and confidence in the data generated is growing, validation of microarray expression trends using a second readout remains a critical requirement. This is especially important if the sample size is too small to allow rigorous statistical analysis. For this purpose, the real time fluorescence based reverse transcriptase polymerase chain reaction (RT-PCR) is generally the method of choice. However, in this issue of *Thorax*, Glare *et al*[10] revisit one of the most stubborn problems associated with all RT-PCR based methods—namely, the choice of a reference gene with which to normalise signals obtained to allow the legitimate comparison between samples and eliminate differences of non-biological origin. One of the most commonly used methods is to normalise against a housekeeping gene because its mRNA levels are thought to remain constant. Using competitive RT-PCR, Glare *et al* provide compelling evidence that mRNA levels of two of the most commonly used housekeeping genes in asthmatic airways—glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin—are, in fact, highly variable and therefore totally unsuitable for normalising the expression levels of potential genes of interest. This study is a welcome addition to a growing body of evidence that mRNA levels of a number of traditional housekeeping genes are not invariable under a variety of experimental and pathological conditions.[11] The evidence is now so strong for samples obtained in vivo that their use should either be discontinued or can only be viewed as valid when appropriate experiments have been performed to confirm that their expression is indeed constant under the experimental conditions of the study.

So what are the alternatives for normalising gene expression data? There are no ideal solutions but, for conventional gene expression studies, the use of total cellular RNA has been proposed as one of the least unreliable methods for data normalisation.[12] Although the use of total RNA levels for normalising expression data derived from patient material still has to be fully validated, recent technical advances for RNA quantitation—including the RiboGreen RNA quantification assay and the Agilent Bioanalyzer which allows RNA quantity and quality assessment in a single step—are likely to prove very useful for studies of human biopsy material with very low RNA yields. Another alternative is to use ribosomal RNA (rRNA) which makes up the bulk of total RNA. Despite reservations regarding changes in expression levels and potential imbalances in rRNA and mRNA fractions between different samples, 18S rRNA has recently been validated for normalising expression levels by quantitative RT-PCR analysis under a number of experimental conditions and is demonstrably more reliable than normalising to housekeeping genes.[11 13] While considering the issue of normalisation, it is also worth pointing out that, regardless of the platform used, uncertainties relating to the use of housekeeping genes for signal normalisation are also relevant to microarray experiments. For oligonucleotide based arrays, the most commonly used approach is to scale or normalise the output data using a transcriptome equivalent strategy (or global normalisation) in order to derive an average intensity for each array with the assumption that the total sum of all transcripts present is similar between samples.

Finally, it is also worth remembering that gene expression studies measure mRNA levels and no more. Since most genes are also highly regulated at the post-transcriptional stage, changes in mRNA levels may not necessarily reflect changes at the protein level. In addition, interpreting expression studies in disease versus control tissue is often confounded by the very dramatic differences in cell populations present within the two types of tissue. Genes which appear to be highly differentially expressed may therefore reflect changes in the cellular composition of the tissue rather than changes in gene expression per se. Additional analysis by conventional immunohistochemistry and/or in situ hybridisation therefore becomes essential when analysing whole biopsy tissue. Similarly, important changes in gene expression may be masked because of dilution of the message. This may be particularly problematic when dealing with biopsy tissue where the disease is confined to a small number of cells within the sample. Recent advances in RNA amplification technology[14] and laser capture microdissection (LCM) to sample individual cell populations within a biopsy sample are proving particularly useful for addressing these potential problems.[2]

In conclusion, we now have the means of monitoring gene expression on a scale which was hard to envisage only 5 years ago. The integration of this technology with rapidly evolving innovations in novel computational tools, public domain data repositories, in combination with the appropriate post-microarray validation experiments is likely to have a major impact on our understanding of complex human disease processes in the future.

Thorax 2002;57:754–756

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REFERENCES

1 Schena M, Shalon D, Davis RW, *et al*. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 1995;270:467–70.
2 Miura K, Bowman ED, Simon R, *et al*. Laser capture microdissection and microarray expression analysis of lung adenocarcinoma reveals tobacco smoking- and prognosis-related molecular profiles. *Cancer Res* 2002;62:3244–50.
3 Zuo F, Kaminski N, Eugui E, *et al*. Gene expression analysis reveals matrilysin as a key regulator of pulmonary fibrosis in mice and humans. *Proc Natl Acad Sci USA* 2002;99:6292–7.
4 Zou J, Young S, Zhu F, *et al*. Microarray profile of differentially expressed genes in a monkey model of allergic asthma. *Genome Biol* 2002;3:research0020.
5 Kaminski N, Allard JD, Pittet JF, *et al*. Global analysis of gene expression in pulmonary fibrosis reveals distinct programs regulating lung inflammation and fibrosis. *Proc Natl Acad Sci USA* 2000;97:1778–83.
6 Mariani TJ, Reed JJ, Shapiro SD. Expression profiling of the developing mouse lung: insights into the establishment of the extracellular matrix. *Am J Respir Cell Mol Biol* 2002;26:541–8.
7 Lee JH, Kaminski N, Dolganov G, *et al*. Interleukin-13 induces dramatically different transcriptional programs in three human airway cell types. *Am J Respir Cell Mol Biol* 2001;25:474–85.
8 Kannan K, Kaminski N, Rechavi G, *et al*. DNA microarray analysis of genes involved in p53 mediated apoptosis: activation of Apaf-1. *Oncogene* 2001;20:3449–55.

9 Khan J, Wei JS, Ringner M, *et al.* Classification and diagnostic prediction of cancers using gene expression profiling and artificial neural networks. *Nature Med* 2001;7:673–9.
10 Glare EM, Divjak M, Bailey MJ, *et al.* β-Actin and GAPDH housekeeping gene expression in asthmatic airways is variable and not suitable for normalising mRNA levels. *Thorax* 2002;57:765–70.
11 Goidin D, Mamessier A, Staquet MJ, *et al.* Ribosomal 18S RNA prevails over glyceraldehyde-3-phosphate dehydrogenase and beta-actin genes as internal standard for quantitative comparison of mRNA levels in invasive and noninvasive human melanoma cell subpopulations. *Anal Biochem* 2001;295:17–21.
12 Bustin SA. Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *J Mol Endocrinol* 2000;25:169–93.
13 Schmittgen TD, Zakrajsek BA. Effect of experimental treatment on housekeeping gene expression: validation by real-time, quantitative RT-PCR. *J Biochem Biophys Methods* 2000;46:69–81.
14 Wang E, Miller LD, Ohnmacht GA, *et al.* High-fidelity mRNA amplification for gene profiling. *Nature Biotechnol* 2000;18:457–9.

Cystic fibrosis

Psychological consequences of segregation resulting from chronic *Burkholderia cepacia* infection in adults with CF

A J A Duff

Patients with CF segregated because of *Burkholderia cepacia* infection must be helped to assemble coherent structures for social relationships if they are to adapt successfully to such management.

In 1997 the median life expectancy for individuals with cystic fibrosis (CF) was 31.5 years in the UK[1] and it has been suggested that those born today can expect to live well into their mid 40s.[2] However, there is huge variability in the physical condition of adults with CF. While malabsorption, osteoporosis, diabetes, and liver failure all contribute to incapacity, lung disease is the main cause of morbidity and mortality. Some patients have near normal levels of lung function. Others, however, are debilitated by dyspnoea and dependent on oxygen.

Lung disease in CF is primarily due to the consequences of infection. In the first decade of life *Staphylococcus aureus* and *Haemophilus influenzae* are the predominant organisms in sputum, while in older children and adults *Pseudomonas aeruginosa* is most common.[3] In the past 15 years some CF centres have had epidemics of *Burkholderia cepacia* infection. Although patients respond to standard antibiotic treatment,[4] most become chronically infected and experience a more rapid decline in lung function. The reasons for this are still unclear, although recent microbiological findings suggest that there are different pathogenic potentials of various *B cepacia* genomovars.[5] In the UK prevalence rates vary between centres but increase significantly if spread from patient to patient is not prevented. In this respect, *B cepacia* differs from other bacteria in that it is usually caught through close or frequent contact with another *B cepacia* positive CF patient.[5 6]

In most UK adult CF centres it is now accepted practice to separate patients who are infected with *B cepacia* from those who are not. Guidelines on cross infection effectively mean managing infected patients in isolation, away from the main CF wards, but even this may not be sufficient to prevent the spread of the organism. Contemporary advice to patients extends segregation to outside hospitals—directing them not to attend CF meetings, not to have any physical contact with *B cepacia* negative CF patients, and to adopt impeccable hygienic behaviour.[7] Although difficult, where this has been done fewer patients with CF become infected with *B cepacia* for the first time[8] and some clinicians now report a decrease in the overall number of cases. However, as a result of the emergence of cross infection in CF patients by a multiresistant strain of *P aeruginosa*, some clinicians now advocate segregating patients according to their microbiological status.[9 10] Others have questioned the wisdom of adopting such practice, highlighting—in addition to clinical reasons—the potential emotional impact on patients and their families.[11]

PSYCHOLOGICAL CONSEQUENCES OF SEGREGATION

While it is known that adults with chronic illness are at a greater risk of developing psychological problems,[12] prevalence rates of such difficulties in adult CF populations are still largely unknown. What is known is that individuals at the end stages of the condition are even more vulnerable to psychological problems resulting from increasing "loss" of, for example, mobility, autonomy, relationships, and quality of life. Furthermore, the disease becomes more "visible" and intrusive and less predictable, which can give rise to feelings of anhedonia, helplessness, fear, and anxiety.[13] With this in mind, while much has been published on the physical benefits of segregating patients with CF, almost no information exists on the psychosocial consequences of such practices.

It is well recognised that being "hospitalised" has a negative effect on psychological functioning.[14–17] Isolation in hospital has the potential to have even greater negative effects on emotional well being. One study reported that over 42% of patients identified negative emotions associated with isolation.[18] Such patients have significantly higher rates of anxiety and depression and significantly lower levels of self-esteem and control.[19] Evidence from other patient groups who have experienced segregated and isolating medical treatments (such as those with cancer, leprosy or HIV positive patients) suggests that the experience is confining, depressing, boring and lonely, leading to feelings of clinical depression, despair and abandonment.[20] Indeed, loneliness, monotony and stigmatisation have been reported as frequently as potential positive aspects of segregation such as having time for reflection, which some patients find very therapeutic.[21] In adult men diagnosed as HIV positive, social isolation is thought to be compounded by ruptures in relationships and the breakdown of social support networks.[22] In addition, while there may be a high desire among patients to receive information and reassurance, being segregated appears to inhibit communication.[18 23] Colonisation with *B cepacia* has resulted in exclusion from CF conferences and support groups, leading to the loss of mutual support systems typically available to adults with CF[24] and, consequently, to further increases in feelings of isolation, anger, and of being a "microbial leper".[25]